

The University of Chicago

RESPONSES OF THE BEAN PLANT TO
CALCIUM DEFICIENCY

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1939

By
HUGH G. GAUCH

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
PLANT PHYSIOLOGY
Vol. 15, No. 1, January, 1940

The University of Chicago

RESPONSES OF THE BEAN PLANT TO
CALCIUM DEFICIENCY

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1939

By
HUGH G. GAUCH

Private Edition, Distributed by
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
PLANT PHYSIOLOGY
Vol. 15, No. 1, January, 1940



RESPONSES OF THE BEAN PLANT TO CALCIUM DEFICIENCY

HUGH GAUCH

(WITH SEVENTEEN FIGURES)

Introduction

Calcium has long been regarded as an essential element for most plants, and many rôles have been assigned to it by numerous investigators. It is, however, beyond the scope of this paper to review all of the literature dealing with calcium, or with each of the many rôles that have been attributed to it. The recent review by MILLER (11) is an excellent source for a general survey of the literature.

Many studies have been made comparing the growth of various plants with and without calcium in the nutrient solution. In many cases, however, the solutions have been chosen arbitrarily without a thorough understanding of the effects produced by various combinations of salts. Experiments with various single salts, two-salt combinations, and various total concentrations of salts were deemed necessary in order to obtain the most favorable plus- and minus-calcium solutions for the growth of the bean plant. The differences between the plants growing in these two solutions are believed to indicate more truly the morphological and chemical alterations that result from a deficiency of calcium in the nutrient solution.

Materials and methods

The plant used in this study was the Dwarf Red Kidney bean, *Phaseolus vulgaris*. The seeds were obtained from a commercial grower of beans at Marysville, California. In these studies the seeds were planted at a depth of one inch in typical greenhouse flats containing unused white, coarse sand, and germinated in darkness for seven days. The sand was kept moist by additions of tap water (containing approximately 32, 11, 1, 0.1, and 15 p.p.m. of calcium, magnesium, potassium, nitrate, and sulphate, respectively). Bean seeds are very sensitive during germination to an over- or an under-supply of

moisture. Temperature in the dark room fluctuated from 78° to 80° F., and the relative humidity varied from 30 to 50 per cent. The seedlings were from seven to eight days old at the time of transfer to the nutrient solutions (fig. 1).



FIG. 1. Seven-day-old bean seedlings as transferred to nutrient solution.

The flats were flooded with tap water to facilitate removal of the seedlings, and after the sand was washed from the roots, the roots were temporarily immersed in distilled water before planting. In each planting, 360 seedlings were accommodated by the 72 one-half gallon glazed crocks, *i.e.*, five plants per crock. The crocks were covered with sheets of cork (paraffined), 6" × 6" × ¼", which served as supports for the plants. Nonabsorbent cotton was pressed lightly into the space between the hypocotyl and the cork support (fig. 4). Early experiments on bean showed that aeration of the cultures was unnecessary for the length of the growth period under study.

As mentioned earlier, many single salts as well as two-salt combinations in various total concentrations were tested; the details of these experiments are reported later. The growth responses—morphological and chemical—are reported in detail for two types of plus- and minus-calcium solutions: (1) those used by NIGHTINGALE, *et al.* (12) (designated "solutions A+ and A-"),

TABLE I

COMPOSITION OF THE PLUS- AND MINUS-CALCIUM SOLUTIONS

SOLUTION AND SYMBOL*	PARTIAL VOLUME-MOLECULAR CONCENTRATION				
	Ca(NO ₃) ₂	KH ₂ PO ₄	MgSO ₄	KNO ₃	NaNO ₃
Nightingale, <i>et al.</i> :					
Plus-calcium (A+)	0.018	0.009	0.009		
Minus-calcium (A-)		0.009	0.009	0.026	
For bean:					
Plus-calcium (B+)	0.0075	0.005	0.0005		
Minus-calcium (B-)		0.005	0.0005		0.015

* Plus 0.5 p.p.m. of boron, as boric acid, and 10 p.p.m. of iron, as ferric citrate.

and (2) solutions adapted to the bean plant (designated "solutions B+ and B-"). Solutions B+ and B- were prepared on the basis of the results obtained from single-salt and two-salt studies covering a wide range of concentrations (table I).

At the time of harvest, the plants were divided into five fractions, *viz.*, root, hypocotyl, cotyledons, primary leaves, and a residual fraction (first internode plus that portion above the primary leaves). The latter fraction is referred to as "first internode and 'top'."

PHYSICAL MEASUREMENTS

Fresh weights of plants, or plant fractions, were determined on a torsion balance sensitive to 0.1 gram. Oven-dry weights were determined on plant material that had been dried at 100° C. in an electric oven for 24 hours, after which it was dried to constant weight in a vacuum oven at 80° C. Oven-dry weights were obtained by use of an analytical balance.

CHEMICAL METHODS

TOTAL NITROGEN.—Total nitrogen was determined by the GUNNING method modified to include the nitrogen of nitrates (7).

ASH DETERMINATIONS.—Duplicate samples of the oven-dry material were ashed to constant weight at a dull-red heat in a muffle furnace.

CALCIUM DETERMINATIONS.—Calcium was determined, with slight modification, according to the method outlined by LINDNER and KIRK (6). After ashing, the sample was dissolved in 1:1 hydrochloric acid, and 10 ml. of ammonium oxalate (saturated) plus two drops of brom-cresol-purple, were added. The mixture was heated just to boiling, and dilute ammonium hydroxide was added until the indicator turned purple. After standing overnight for precipitation, the mixture was filtered and the precipitate of calcium oxalate was washed with dilute (10 per cent.) ammonium hydroxide, saturated with calcium oxalate. A special type of sintered-glass filter was used in order that the final titration of the oxalic acid might be carried out in the same Erlenmeyer flask in which the calcium oxalate was precipitated, and hence minimized any loss. The calcium oxalate was dissolved in 2 N sulphuric acid, heated on a hot plate, and 20 ml. of 0.01 N (or 0.1 N, with the larger amounts of calcium) ceric sulphate were added before setting aside to cool. The excess ceric sulphate, that remained after reacting with oxalic acid, was titrated with 0.01 N ferrous ammonium sulphate, using the ortho-phenanthroline-ferrous complex as the indicator.

Experimentation

SINGLE-SALT STUDIES

In order that the effect of a single salt alone might be determined, plants were grown in various single-salt solutions over a wide range of concentra-

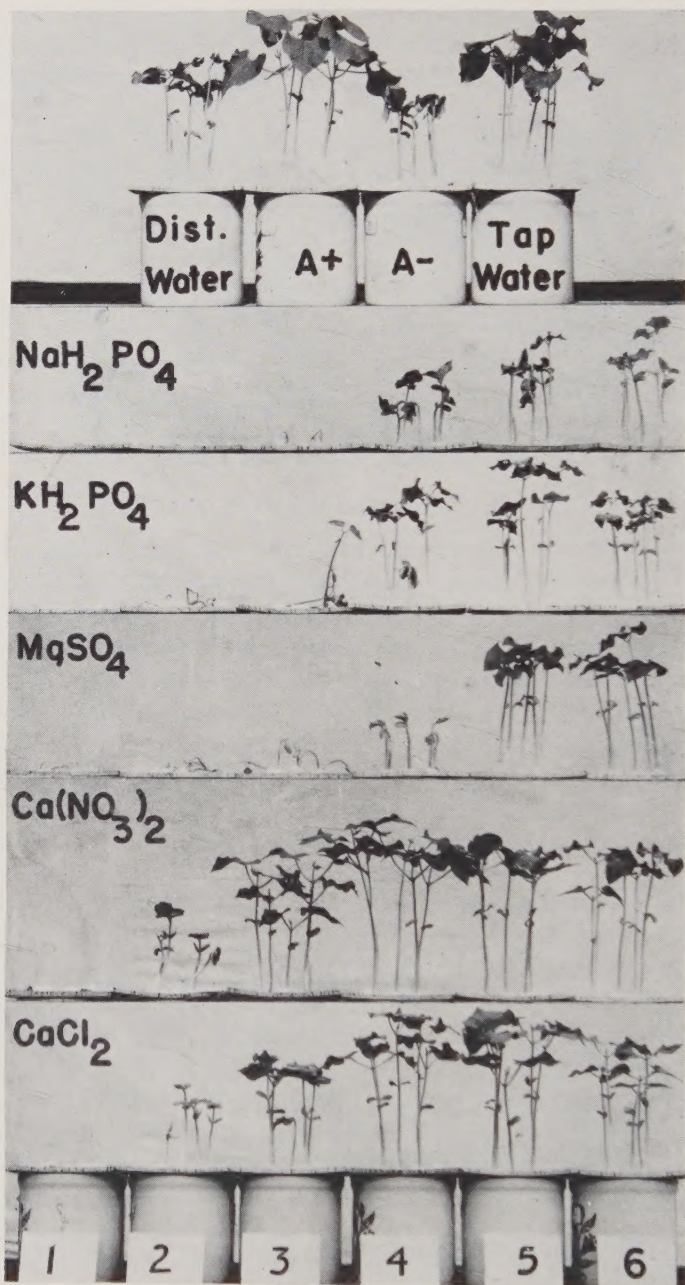


FIG. 2. Bean plants, 9 days after planting into various concentrations of single-salt solutions. Concentrations, vertical rows 1 to 6, are: 0.5, 0.1, 0.05, 0.01, and 0.001 M.

tions, including 0.5, 0.1, 0.05, 0.01, 0.001, and 0.0001 molar. $\text{Ca}(\text{NO}_3)_2$, CaCl_2 , MgSO_4 , MgCl_2 , KNO_3 , KCl , KH_2PO_4 , NaNO_3 , NaCl , NaH_2PO_4 , and Na_2SO_4 were used. For all except the two calcium salts, 0.1 and 0.5 M proved to be too concentrated, while the response of plants in the 0.0001 M concentration was essentially like that produced by distilled water (fig. 2). With the exception of Na_2SO_4 , which was very toxic, responses in the sodium and potassium salts were much alike. Representative salts of these two cations are shown in figure 2.

High amounts of potassium and sodium produce similar, characteristic symptoms. With an excess of either of these two cations, the hypocotyl becomes more or less water-soaked in appearance and assumes a light tan color.

Little or no root growth resulted in any solutions except those of the calcium salts. With the magnesium salts there was a typical burning of the pulvinus at the base of the primary leaves, and spotting and necrosis of the leaf blades. Moreover, by the second day after planting, the hypocotyls were a very deep, reddish-brown color in the 0.5, 0.1, and 0.05 M concentrations. The coloration first appeared opposite the protoxylem points, and then soon spread throughout the hypocotyl tissue in general.

TWO-SALT COMBINATIONS

After determining the effects produced by a number of single salts, two-salt combinations were studied. The three combinations that were studied dealt with $\text{Ca}(\text{NO}_3)_2$ and MgSO_4 , $\text{Ca}(\text{NO}_3)_2$ and KH_2PO_4 , and KH_2PO_4 and MgSO_4 . Each salt in varying amounts (0.05, 0.5, 2.5, 5, 12.5, and 25 ml. of molar salt per liter of solution) was combined separately with each of the other two salts. This method of combining these salts yields 108 different solutions. Dry weight of the tops, minus cotyledons, was taken as the criterion of growth. If one considers the average growth produced by the various amounts of MgSO_4 or KH_2PO_4 , when plotted against $\text{Ca}(\text{NO}_3)_2$, on the abscissa, 5 to 12.5 ml. of molar $\text{Ca}(\text{NO}_3)_2$ per liter of solution is the optimal amount (fig. 3A). Combining MgSO_4 with KH_2PO_4 (fig. 3B), 5 ml. molar KH_2PO_4 per liter is optimal; 2.5 to 5 ml. is the optimal amount of molar KH_2PO_4 when combined with $\text{Ca}(\text{NO}_3)_2$ (fig. 3B). When KH_2PO_4 is combined with MgSO_4 (fig. 3C), 0.05 ml. of molar MgSO_4 per liter is suboptimal, 0.5 ml. is optimal, and greater amounts of MgSO_4 result in progressively less growth of the plants. Also of interest is the fact that with the optimal amount, or less, of MgSO_4 there is no magnesium-burning or toxicity. When $\text{Ca}(\text{NO}_3)_2$ is combined with MgSO_4 , 0.05 ml. of molar MgSO_4 per liter is also limiting, while 0.5 ml. gives optimal and maximal dry weight of plants. Thus it is shown that when MgSO_4 is combined with a calcium salt the higher amounts (above optimum) of MgSO_4 can be tolerated by the plants with no decrease in the amount of growth. This is not true for the combination of KH_2PO_4 with MgSO_4 (fig. 3C).

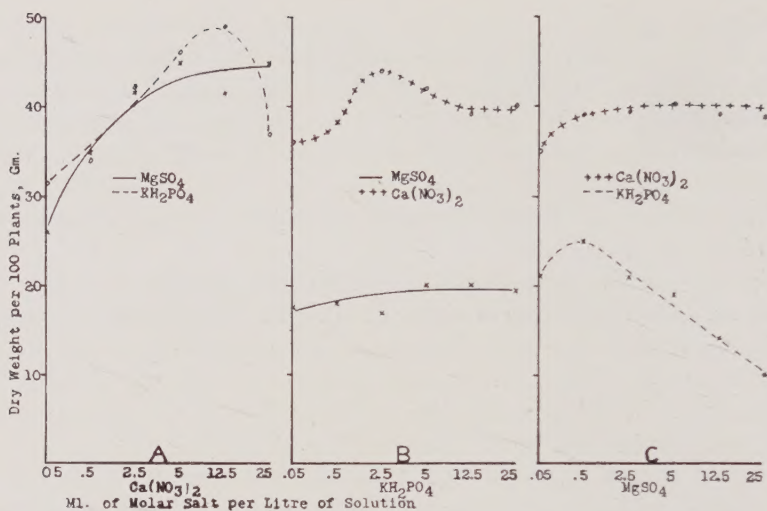


FIG. 3. Growth of bean plants in two-salt solutions. Each curve represents the average growth produced by the various concentrations of a given salt when combined with the salt shown on the abscissa.

On the basis of the results from the single-salt solutions, the two-salt combinations, and the dilution studies (in the following section), the B+ and B- solutions were compounded.

DILUTION STUDIES

The A+ and A- solutions, *i.e.*, 2 atmospheres, and various dilutions of

TABLE II

SIX-DAYS' GROWTH OF BEAN PLANTS IN VARIOUS CONCENTRATIONS OF THE A+ AND A- SOLUTIONS

CONCENTRATION OF SOLUTION	OVEN-DRY WEIGHT PER 100 PLANTS	
	TOPS*	ROOTS
<i>atm.</i>	<i>gm.</i>	<i>gm.</i>
A+:		
2.0	35.5	4.4
1.0	42.0	5.7
0.1	38.4	5.5
0.01	30.6	5.4
0.001	24.6	3.4
Tap water	30.0	5.3
A-:		
2.0	18.0	0.9
1.0	19.3	1.3
0.1	28.0	2.9
0.01	26.4	3.8
0.001	24.0	3.3
Distilled water	26.6	3.0

* Minus cotyledons.

them (to 1, 0.1, 0.01, and 0.001 atm.) were used, with tap water and distilled water as controls. Dry weights of the tops and roots were taken as the criteria of growth. The results are shown in table II and figure 4.

It is of interest to note that in the A- series the greatest dry weight of tops occurred in the 0.1-atm. concentration. In this concentration the magnesium content is 1/20 of the amount present in the regular A- solution. With a concentration of 0.1 atm. the leaves of the plants did not show magnesium



FIG. 4. Bean plants, 5 days after planting, in various dilutions of the A+ and A- solutions. Concentrations are: 2, 1, 0.1, 0.01, and 0.001 atm., crocks 1 to 5; crock 6 is tap water (above), and distilled water (below).

toxicity. This was evidence additional to that obtained in the single- and two-salt studies, suggesting that the magnesium content of a minus-calcium solution should be approximately 0.5 ml. of molar MgSO_4 per liter of solution. This amount of MgSO_4 was used, therefore, in the B+ and B- solutions as applied to bean plants during the stage of development under study. Other stages of development might very likely require different amounts of magnesium and other ions, as illustrated for wheat by McCALL and RICHARDS (8).

INTERRELATION OF CALCIUM AND MAGNESIUM

Inasmuch as it is impossible to designate a single value for the Ca/Mg ratio that will prove "best" in all combinations or all concentrations of salts, it seems preferable to speak of the interrelation of calcium and magnesium, potassium, or other ions. The word "ratio" is convenient, however, for the purpose of expressing the relative amounts of one ion to another, and it will be used in that sense only.

In the present study of the interrelation of calcium and magnesium, KH_2PO_4 and KNO_3 were kept constant at 9 and 26 ml. of molar salt per liter, respectively, while $\text{Ca}(\text{NO}_3)_2$ and MgSO_4 were varied to produce Mg/Ca

ratios of 1, 10, 20, 30, 40, and 50. In one series there was 0.18 ml. of molar $\text{Ca}(\text{NO}_3)_2$ per liter of solution, and the MgSO_4 was varied to produce the above-stated ratios. In another series $\text{Ca}(\text{NO}_3)_2$ was at 1.8 ml.; in still another, at 18 ml. of molar salt per liter of solution, the MgSO_4 being varied accordingly. Thus, although the same set of ratios existed in each of the three series, the total concentration of the series varied, as did also the concentration of the members within a series.

A given ratio of Mg/Ca is not the only factor that will determine plant response, as is illustrated in figure 5. Most of the plants in row C were killed by the high concentrations of salts. In row B the various heights of plants in the different crocks are apparently related to the various concentrations. There was little or no magnesium injury in rows A and B with Mg/Ca ratios of 10 or less. This was more obvious in the case of row B than in A, showing that the effect of ratios may be altered by the total concentration of salts. According to OSTERHOUT (13), growth in concentrated (0.1 M) solutions furnishes a much better criterion of antagonism than growth in dilute solutions; at great dilution about the same amount of growth is obtained regardless of the proportions of the two salts. For wheat, TOTTINGHAM (17) and SHIVE

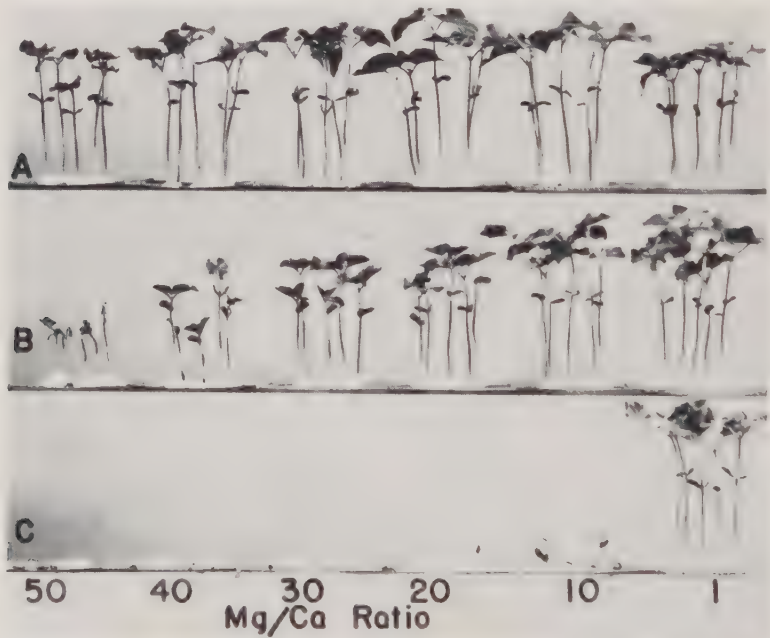


FIG. 5. Responses of the bean plant to the same series of Mg/Ca ratios, but in three different total concentrations of salts. Seven days after planting. Rows A, B and C contain 0.18, 1.8, and 18 ml. of molar $\text{Ca}(\text{NO}_3)_2$ per liter, respectively.

(15) report that the Mg/Ca ratio must be 2, or less, to avoid magnesium injury. It is of interest that a Mg/Ca ratio of 1.0 was not as favorable for the growth of bean plants as the higher ratios. TOTTINGHAM (17) has reported that high concentrations of $\text{Ca}(\text{NO}_3)_2$, in a series of solutions with two per cent. (8.15 atm.) concentrations, produced characteristic and serious injury to the tops of wheat plants.

TRELEASE and TRELEASE (18) state that magnesium injury to wheat, while mainly controlled by the Mg/Ca ratio, appears to be influenced by the proportions and concentrations of other materials present in the culture solution, as well as by the total concentration of the solution and possibly also by climatic conditions. They also report that, between the limits of one and 50 of this ratio, the dry weight of tops of wheat plants is inversely proportional to the log of the Mg/Ca ratio. TRUE and BARTLETT (19) found that absorption by pea roots is greatest in equimolecular proportions of calcium and magnesium nitrates, but that root growth is equally good in any solution which does not contain less than one molecule of calcium to nine of magnesium. In the more dilute solutions, an even lower ratio of Ca/Mg, 1:99, overcame the effects of magnesium alone. GARNER, *et al.* (5), state that it is difficult to distinguish between magnesium toxicity symptoms and minus-calcium symptoms. They found that, in the case of tobacco, there must be present in the leaf 0.25 per cent. of magnesium and 1.0 per cent. of calcium to prevent deficiency symptoms of these cations.

BARBIER (1), working with maize, oats, onion, spinach, and tomato reported that the uptake of an element is a function of its concentration, and concluded that antagonism is relatively unimportant and should not be exaggerated. Similarly, in studies with corn, BECKENBACH, WADLEIGH, and SHIVE (2) observed that none of the three ratios—Ca/Mg, K/Mg, or K/Ca—showed any significance.

This study indicates that there is no well-defined Ca/Mg "ratio" that is optimal for growth over a range of total concentrations of salts, and that it is a matter of interrelationships among calcium, magnesium, and other ions of the solution.

INTERRELATIONSHIP OF CALCIUM AND POTASSIUM

In another series, similar to the Mg/Ca series, calcium and potassium were varied, while MgSO_4 was constant at 9 ml. of molar salt per liter of solution. Calcium nitrate was used at three levels of nutrition, just as in the previous experiment, and the two potassium salts (KNO_3 and KH_2PO_4) were varied to produce K/Ca ratios of 200, 150, 100, 50, 10, and 1. Within 7 days, the appearance of the plants was as shown in figure 6. Rows B and C show growth responses that were apparently associated with concentration, *i.e.*, total concentrations become less from left to right. There was no magnesium burning

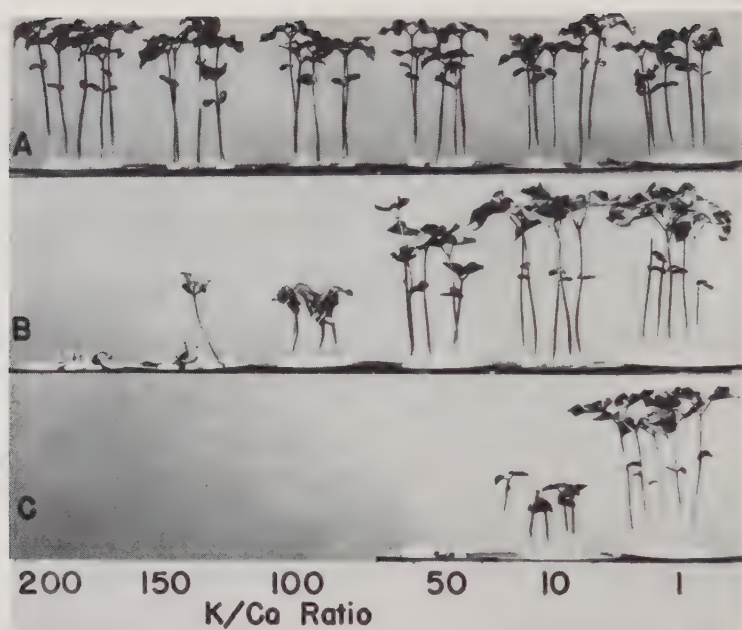


FIG. 6. Responses of the bean plant to the same series of K/Ca ratios, but in three different total concentrations of salts. Seven days after planting. Rows A, B, and C, contain 0.18, 1.8, and 18ml. of molar $\text{Ca}(\text{NO}_3)_2$ per liter, respectively.

in either row B or C. In row B there was no burning, and the ratio of Mg/Ca was only five. In row A the plants were of similar heights and growth-form, but all of the plants in this row were severely burned owing to the high Mg/Ca ratio of fifty.

According to McCALLA and WOODFORD (9), limiting the amount of one element in a nutrient brings about an increase in absorption of another ion of the same sign, or a decrease in absorption of all ions of the opposite sign. Limitation of potassium is accompanied by an increase in the absorption of both calcium and magnesium. Limitation of calcium results in a relative increase in the absorption of both potassium and magnesium, with the increase in magnesium being considerably the greater.

Thus it seems that calcium plays an important rôle within the cells and in the so-called physiological balance of nutrient solutions by overcoming, within certain limits, the toxic effects of too high a concentration of any other cation. This interrelation of the various cations is not a static condition; it is dynamic and continually varying—depending upon the relative concentrations of the cations present. These interrelations of cations are presumably effective in part through their action on the colloidal properties of protoplasm.

COMPARISON OF THE A+ AND A- SOLUTIONS WITH THE
B+ AND B- SOLUTIONS

Calcium deficiency affects the terminal bud, leaves, and roots of the bean plant in much the same way as reported for tobacco by McMURTREY (10).

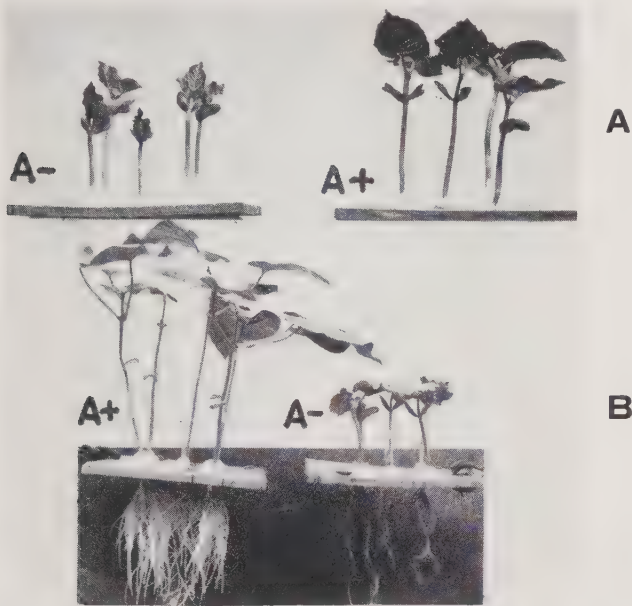


FIG. 7. Growth of bean plants in the A+ and A- solutions. A, three, and B, seven days after planting.

Plants growing in the A- solution (fig. 7) are short (stunted) with small, wrinkled leaves. They die within six to eight days from the time of transfer. The dry weight (fig. 9) and total nitrogen (fig. 13) do not increase from the second to the sixth day. Ash constituents (fig. 10) continue to enter these plants, but nitrates do not. This is in accordance with the findings of NIGHTINGALE, *et al.* (12), for tomato. The calcium content (fig. 14) increases slightly and then it decreases. The fact that the roots of these plants contain relatively large amounts of calcium indicates that the calcium, although entering in very small amounts, is bound in the roots. In general the dry weight (fig. 11), ash (fig. 12), nitrogen (fig. 15), and calcium (fig. 16) decrease only slightly in the cotyledons of plants growing in the A- solution.

On the other hand, in the B- solution (fig. 8) the dry weight (fig. 9), ash (fig. 10), and total nitrogen of the plants (fig. 13) increase markedly until after the eighth day. The plants live for two weeks or more from the time of transfer, are taller, have larger leaves, and wrinkling, or necrosis, of the



FIG. 8. Growth of bean plants in the B⁺ and B⁻ solutions. A, four, B, eight, and C, twelve days after planting.

leaves is less pronounced or nearly absent. Ash and total nitrogen increase at approximately the same rate. Despite the fact that the B- solution is a minus-calcium solution, nitrates are absorbed to as great an extent as are the ash constituents. This is in contradiction to the statement by NIGHTIN-

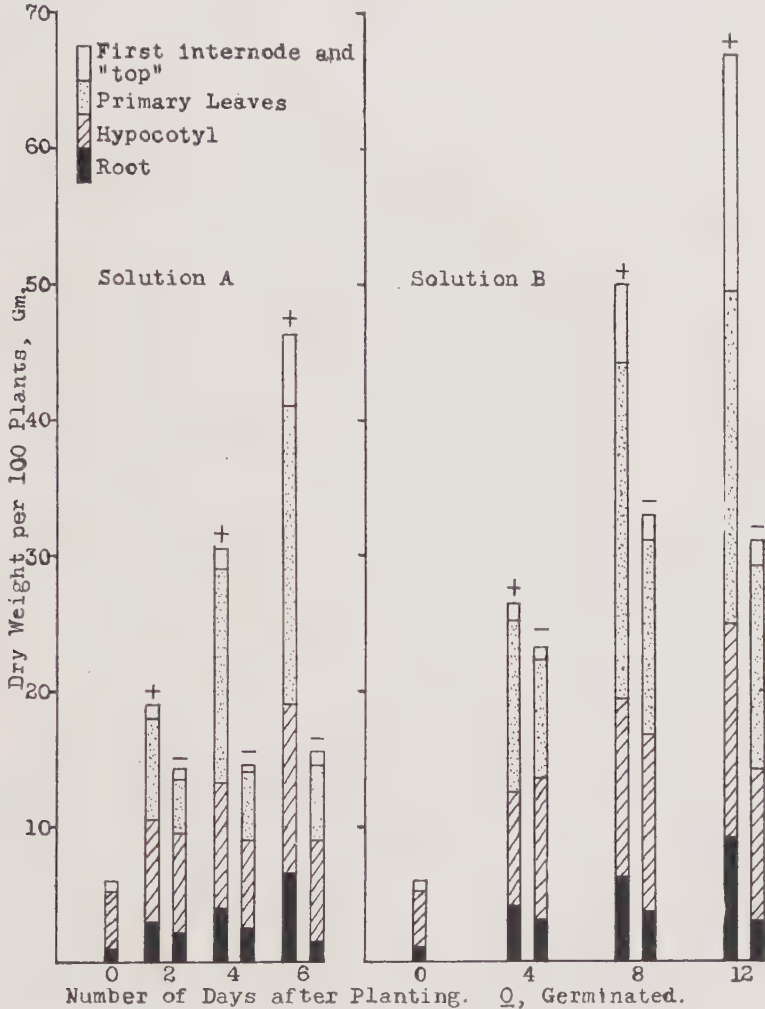


FIG. 9. Dry weight of bean plants.

GALE, *et al.* (12), that minus-calcium plants cannot absorb nitrates. It is true that they do not do so in the A- solution, but they do in the B- solution. It seems likely, therefore, that the composition of the A- solution is such that the unbalance in the absence of calcium is responsible for the nonabsorption of nitrates. As mentioned earlier in this paper, the amount of magnesium

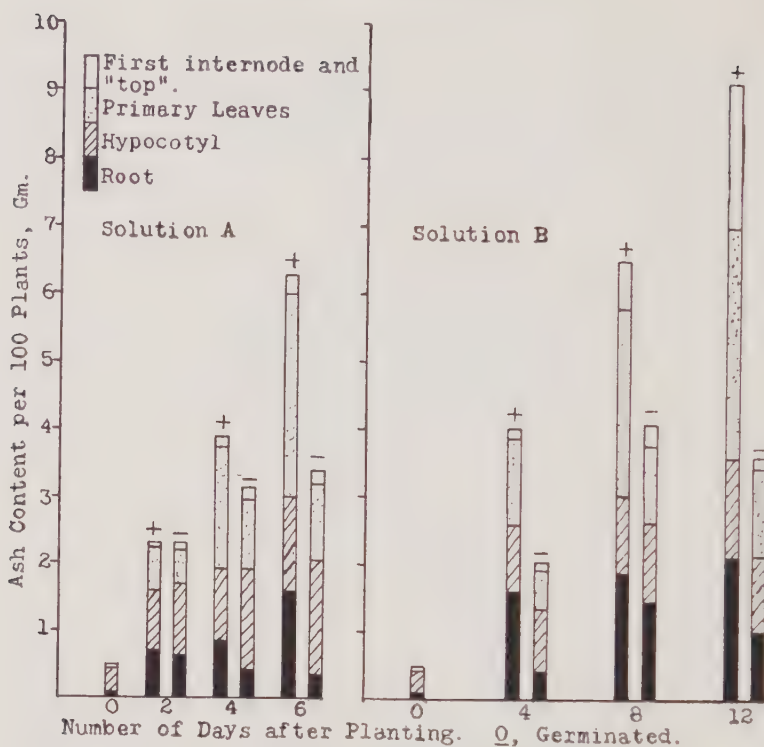


FIG. 10. Ash content of bean plants.

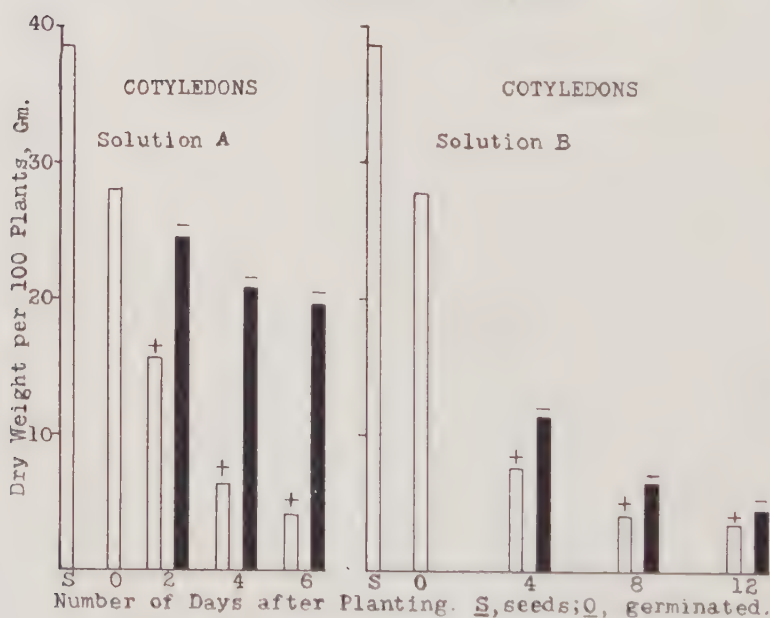


FIG. 11. Dry weight of cotyledons of bean plants.

appears to be one of the main disturbing factors in the A- solution. Inasmuch as the nitrogen content of plants in the B- solution increased markedly, while the calcium content remained more or less constant and low, there is apparently not necessarily a close relationship between calcium and nitrogen contents, as proposed by PARKER and TRUOG (14). There may be a correlation, however, between the utilization of nitrogen and the content of calcium. The latter relationship was not determined in this study, but ERMAKOV (4) and BURRELL (3) have reported that, associated with calcium deficiency, there is an accumulation of nitrates in leaves owing to the fact that nitric nitrogen is not assimilated and converted to organic forms of nitrogen.

In the B- solution, the dry weight (fig. 11), ash (fig. 12), and nitrogen (fig. 15) of the cotyledons decrease to about the same amounts and at the same rate as in the plants of the B+ solution. The calcium content (fig. 16) of cotyledons increases in plants growing in the plus-calcium solutions, and tends to remain more or less constant in the A- or B- solutions. In the absence of sufficient calcium in the nutrient solution, the reserve calcium of the cotyledons is apparently unavailable.

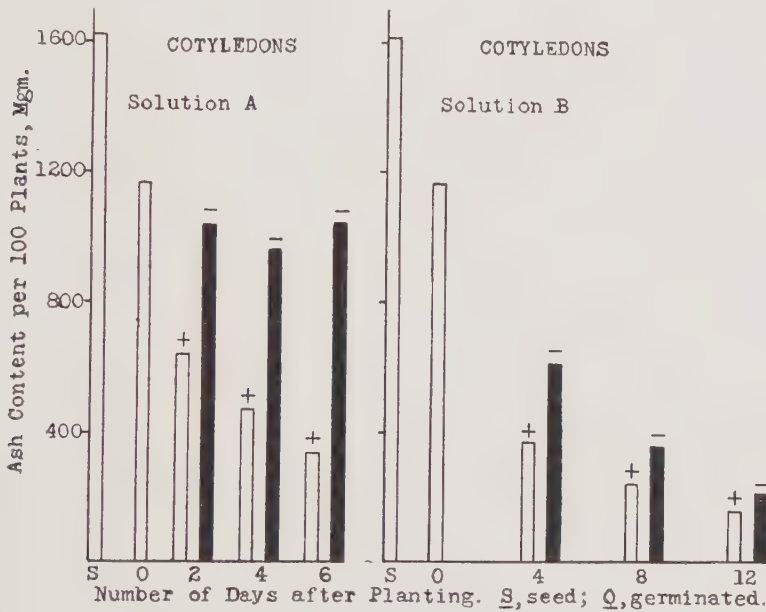


FIG. 12. Ash content of cotyledons of bean plants.

The hypocotyls of plants growing in the A- solution contain more ash than the hypocotyls of plants in the A+ solution, as shown in figure 10. On the other hand, in the B- solution in which nitrates are readily absorbed, the hypocotyls of plants growing in that solution contain more nitrogen (fig. 13) than the hypocotyls of plants in the B+ solution. The reason for this

directional shift of ash and nitrogen to the hypocotyls is unknown, but it is of interest that a similar shift of such materials occurs when the cut surfaces of bean hypocotyls are treated with indoleacetic acid, as reported by STUART

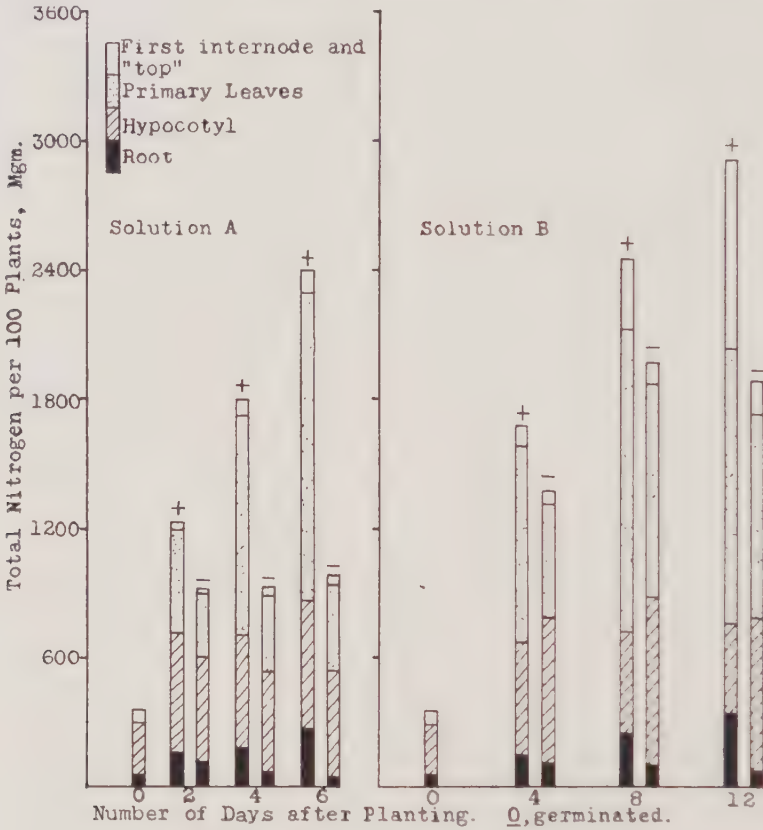


FIG. 13. Total nitrogen content of bean plants.

(16). In the response of the bean plant to a deficiency of calcium, including the degeneration of the original root system, there is a tendency toward the production of adventitious roots, especially near the base of the hypocotyl. Such roots do not reach more than one or two millimeters in length.

GROWTH RESPONSES FOLLOWING TRANSFER OF PLANTS BOTH FROM THE B- TO THE B- SOLUTION AND FROM THE B- TO THE B+ SOLUTION

Each day, for 12 days after planting, plants were transferred from the B- to the B+ solution; plants were similarly transferred from the B+ to the B- solution. As shown in figure 17, the earlier that B- plants were placed on B+ nutrient, the more completely did they resemble B+ plants, and the better was their recovery in general. If transferred between the twelfth and

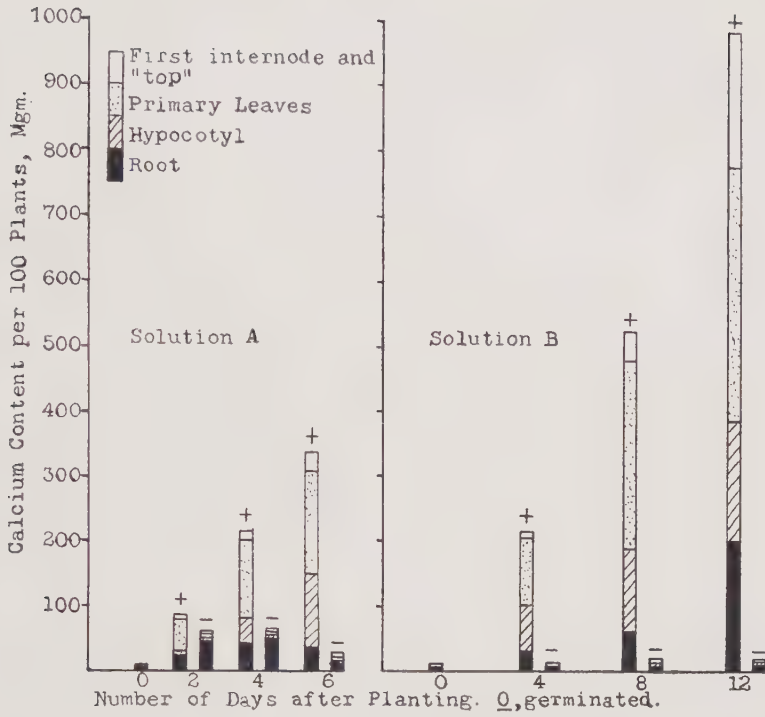


FIG. 14. Calcium content of bean plants.

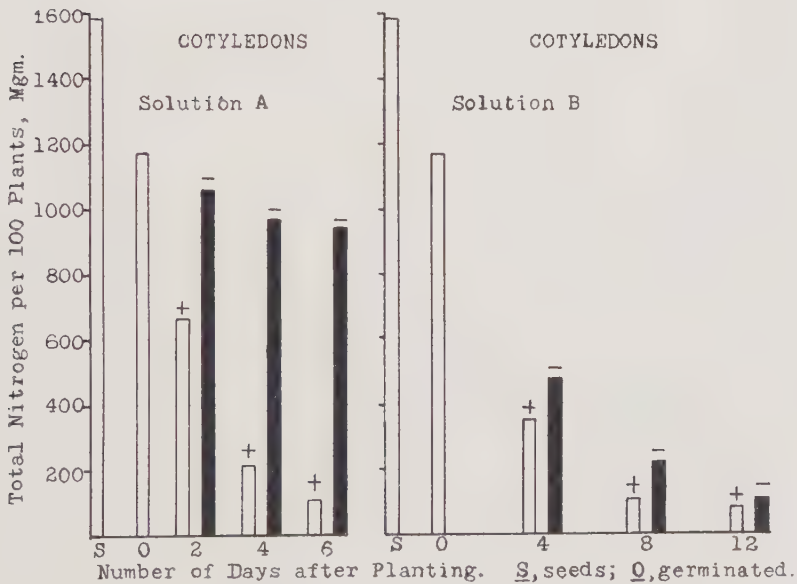


FIG. 15. Total nitrogen content of cotyledons of bean plants.



FIG. 16. Calcium content of cotyledons of bean plants.

fourteenth days, the axillary buds of the primary leaves finally grew out, but the terminal bud was dead, as judged from its appearance and from the fact that it did not grow. It seems probable that the terminal bud was originally the most active of the three buds, and that the absence of calcium affected its metabolism first, causing its death before that of the axillary buds. The original roots were dead on the B- plants transferred after six days, but new adventitious roots arose from the bases of the hypocotyls up to and including at least the twelfth day.



FIG. 17. Response of bean plants after transfer from B- to B⁺, and from B⁺ to B- solution. Photograph taken on 13th day after day of planting.

When B+ plants were transferred to B- solution at similar intervals, root growth soon ceased, especially in those B+ plants transferred early into B- solution. Within 10 to 12 days after the second- and fourth-day transfers had been made, the petioles of the primary leaves became weak and the leaves pendant (fig. 17). The cause of this response is unknown, but it approximated the symptoms of high potassium or high sodium. During the first four days after planting, there was a large increase in the amount of ash (fig. 10). In the absence of calcium, apparently relatively greater amounts of other cations are absorbed. With limitation of calcium, McCALLA and WOODFORD (9) observed a relative increase in the absorption of both potassium and magnesium, with the increase in magnesium being considerably the greater. In the B- solution, the total magnesium content is low, but, in this instance, relatively greater amounts of sodium may have entered; the latter element acts, within the plant, much the same as does potassium.

Summary

1. Responses of the bean plant to various concentrations of single salts are reported.

2. From the results obtained in the single-salt studies, various two-salt combinations were made, and the responses of the plants growing therein are reported.

3. On the basis of those and other studies, plus- and minus-calcium solutions (solutions B+ and B-) especially adapted to bean were devised in which there is no apparent excess or deficiency of essential ions other than calcium.

4. The responses of bean plants to two types of solutions were studied, *viz.*, (a) NIGHTINGALE'S, *et al.*, plus- and minus-calcium solutions (solutions A+ and A-), and (b) plus- and minus-calcium solutions adapted to bean (solutions B+ and B-).

5. Bean plants growing in the A- solution continue to absorb ash constituents, but they do not absorb nitrates. This is in accordance with the findings of NIGHTINGALE, *et al.*, for tomato.

6. Plants growing in the B- solution do, however, absorb appreciable quantities of nitrates, and the increases in dry weight, total nitrogen, and ash of these plants are parallel.

7. Dry weight, total nitrogen, and ash of the cotyledons from plants growing in the B- solution decrease markedly in amount, while those fractions decrease only very slightly when the plants are grown in the A- solution.

8. Bean plants, with cotyledons intact, live approximately six days in the A- solution, and twelve days in the B- solution.

9. There appear to be no ratios, *e.g.*, Ca/Mg or Ca/K, that are optimal for growth, over a range of concentrations of salts.

The writer is indebted to members of the Department of Botany of the University of Chicago for helpful suggestions made during the course of this study.

THE UNIVERSITY OF CHICAGO

LITERATURE CITED

1. BARBIER, G. The mineral nutrition of the plant as a function of the chemical composition of its medium. *Ann. Agron.* **6**: 568-586. 1936.
2. BECKENBACH, J. R., WADLEIGH, C. H., and SHIVE, J. W. Nutrition studies with corn: 1. A statistical interpretation of the nutrient-ion effect upon growth in artificial culture. *Soil Sci.* **41**: 469-489. 1936.
3. BURRELL, R. C. Effect of certain deficiencies on nitrogen metabolism of plants. *Bot. Gaz.* **82**: 320-328. 1926.
4. ERMAKOV, V. P. Relation of the salts of calcium to the assimilation of nitrate nitrogen by green plants. *Univ. Izv. (Kiev)* **48**: 1-68; abs. in *Zhur. Opuitn. Agron. (Russ. Jour. Expt. Landw.)* **10**: 394-396. 1908.
5. GARNER, W. W., McMURTREY, J. E. JR., BOWLING, J. D. JR., and MOSS, E. G. Magnesium and calcium requirements of the tobacco crop. *Jour. Agr. Res.* **40**: 145-168. 1930.
6. LINDNER, R., and KIRK, P. L. Quantitative drop analysis. VII. Further investigations on the determination of calcium. *Mikrochemie* **22**: 291-300. 1937.
7. LOOMIS, W. E., and SHULL, C. A. *Methods in plant physiology.* McGraw-Hill Book Co. New York, 1937.
8. MCCALL, A. G., and RICHARDS, P. E. Mineral food requirements of the wheat plant at different stages of its development. *Jour. Amer. Soc. Agron.* **10**: 127-134. 1918.
9. MCCALLA, A. G., and WOODFORD, E. K. Effects of a limiting element on the absorption of individual elements and on the anion:cation balance in wheat. *Plant Physiol.* **13**: 695-712. 1938.
10. McMURTREY, J. E. JR. Distinctive effects of the deficiency of certain essential elements on the growth of tobacco plants in solution cultures. *U. S. Dept. Agr. Tech. Bull.* 340. 1933.
11. MILLER, E. C. *Plant physiology.* 2nd ed. McGraw-Hill Book Co. New York, 1938.
12. NIGHTINGALE, G. T., ADDOMS, R. M., ROBBINS, W. R., and SCHERMERHORN, L. G. Effects of calcium deficiency on nitrate absorption and on metabolism in tomato. *Plant Physiol.* **6**: 605-630. 1931.
13. OSTERHOUT, W. J. V. The forms of antagonism curves as affected by concentration. *Bot. Gaz.* **58**: 367-371. 1914.

14. PARKER, F. W., and TRUOG, E. The relation between the calcium and the nitrogen content of plants and the function of calcium. *Soil Sci.* **10**: 49-56. 1920.
15. SHIVE, J. W. A study of physiological balance in nutrient media. *Physiol. Res.* **1**: 327-397. 1915.
16. STUART, N. W. Nitrogen and carbohydrate metabolism of kidney bean cuttings as affected by treatment with indoleacetic acid. *Bot. Gaz.* **100**: 298-311. 1938.
17. TOTTINGHAM, W. E. A quantitative chemical and physiological study of nutrient solutions for plant cultures. *Physiol. Res.* **1**: 133-245. 1914.
18. TRELEASE, S. F., and TRELEASE, HELEN M. Magnesium injury of wheat. *Bull. Torrey Bot. Club* **58**: 127-148. 1931.
19. TRUE, R. H., and BARTLETT, H. H. Absorption and excretion of salts by roots, as influenced by concentration and composition of culture solutions. I. Concentration relations of dilute solutions of calcium and magnesium nitrates to pea roots. U. S. Dept. Agr. Bur. Plant Ind. Bull. 231. 1912.

